Criteria Specification

ClinGen Monogenic Diabetes Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for HNF4A Version 1.0.0

Affiliation: Monogenic Diabetes VCEP
Description : ClinGen Monogenic Diabetes Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines
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Rules for HNF4A

Gene: HNF4A (HGNC:5024)
Preferred Transcript: NM_175914.5
HGNC Name: hepatocyte nuclear factor 4 alpha
Disease: monogenic diabetes (MONDO:0015967)

Criteria & Strength Specifications

PVS1

Original ACMG Summary

Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.
Caveats:
• Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7).
• Use caution interpreting LOF variants at the extreme 3’ end of a gene.
• Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.
• Use caution in the presence of multiple transcripts.

Very Strong

Use *HNF4A* PVS1 decision tree.

- Variants generating PTCs in exon 10 and last 55 nucleotides of exon 9 (c.1162-1216) are not expected to cause NMD[1]
    - The most 3’ nonsense or frameshift variant is c.1256C>G, p.S419X in the last exon. This variant has been classified as Pathogenic by the MDEP. There are six other nonsense and frameshift variants in exon 10, none of which have case information and are all currently classified as VUS. The collective evidence supports applying PVS1 for variants at codon 419 (c.1257) and 5’ and PVS1_Supporting for variants at c.1258 (G)/p.Gly420 and 3’.

- “Exon skipping or use of a cryptic splice site that preserves reading frame” and “Single to multi-exon deletion that preserves reading frame”
  - Exons 1, 2 (LRG 4), 3 (LRG 5), 4 (LRG 6), 6 (LRG 8): deletion or skipping causes frameshift: PVS1
  - Exons 5 (LRG 7), 7 (LRG 9), 8 (LRG 10), 9 (LRG 11) - deletion or skipping causes in-frame deletion, 52/52/79/51-79 AA deleted, that is >10 % of the protein in each case - PVS1_Strong
  - Exon 10 (LRG 12) - 46 AA, contains the transactivation domain, includes stop loss - PVS1_Strong

Modification Type: Strength

Strong

Use *HNF4A* PVS1 decision tree.

- “Exon skipping or use of a cryptic splice site that preserves reading frame” and “Single to multi-exon deletion that preserves reading frame”
  - Exons 5 (LRG 7), 7 (LRG 9), 8 (LRG 10), 9 (LRG 11) - deletion or skipping causes in-frame deletion, 52/52/79/51-79 AA deleted, that is >10 % of the protein in each case - PVS1_Strong
  - Exon 10 (LRG 12) - 46 AA, contains the transactivation domain, includes stop loss - PVS1_Strong

Modification Type: Strength

Moderate

Use *HNF4A* PVS1 decision tree.

Apply PVS1_Moderate to initiation codon variants. MDEP has classified two start codon variants as likely pathogenic (c.3G>A: PM2_Supporting + PP4_Moderate + PP1_Strong + PVS1_Moderate (c.1delA); c.1delA: PM2_Supporting + PP1 + PP4_Moderate + PVS1_Moderate) and there are multiple P/LP variants before the next methionine, p.Met71.

Modification Type: Strength

Supporting

Use *HNF4A* PVS1 decision tree.

Apply PVS1_Supporting to nonsense or frameshift variants at c.1258 (G)/p.Gly420 and 3’.

Modification Type: Strength

Instructions: Per recommendations from the SVI, when RNA analysis demonstrates abnormal splicing from non-canonical splice site variants, apply PS3

instead of PVS1.

PS1

Original ACMG Summary

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.
Example: Val->Leu caused by either G>C or G>T in the same codon.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

No change

Modification Type: No change

Supporting

PS1 may also be used at a supporting level for canonical and non-canonical splicing variants when a different variant at the same nucleotide has been previously classified as pathogenic and the variant being assessed is predicted by SpliceAI to have a similar (SpliceAI score within 10% of the original variant) or greater deleterious impact.

Modification Type: Strength

PS2

Original ACMG Summary

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.
Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

Very Strong

Use SVI recommended point-based system with specifications for “Phenotype Consistency” per instructions.

Modification Type: Gene-specific,Strength

Strong

Use SVI recommended point-based system with specifications for “Phenotype

Consistency" per instructions.

Modification Type: Gene-specific,Strength

Moderate

Use SVI recommended point-based system with specifications for "Phenotype Consistency" per instructions.

Modification Type: Gene-specific,Strength

Supporting

Use SVI recommended point-based system with specifications for "Phenotype Consistency" per instructions.

Modification Type: Gene-specific,Strength

Instructions: To obtain maximum points ("phenotype highly specific for gene") patient must meet criteria for PP4 (result of ≥50% chance or higher of testing positive for MODY on the MODY Probability calculator (https://www.diabetesgenes.org/mody-probability-calculator/) and negative HNF1A testing). To obtain standard points ("phenotype consistent with gene but not highly specific"), the phenotype of the patient must include diabetes. Probands (and/or family members when assessing segregation for PP1) with evidence of an autoimmune etiology of diabetes and/or absolute or near-absolute insulin deficiency will be excluded when assessing criteria that includes phenotype information. Such evidence includes the following:One or more positive diabetes autoantibodies (IA-2A, ZnT8A+, GAD) (Ref 7,8,9,10). Very low or negative C-peptide, defined as either fasting or non-fasting random C-peptide (<200pmol/L or 0.6ng/mL)(Ref 11,12) or urinary C-peptide/creatinine ratio <0.2 nmol/mmol (Ref 8,9)

PS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Strong

Applicable to non-canonical splice site variants that have RNA and in silico evidence of

aberrant splicing.

Modification Type: Gene-specific,Strength

Supporting

List of approved functional studies and guidelines for interpretation:

- EMSA for DNA binding
  - “Decreased function” is defined as activity less than 60% of wildtype
  - Note: the effect of the variant on DNA binding will be highly dependent on whether the variant is located within the DNA binding domain.
- Luciferase assays for transactivation
  - “Decreased function” is defined as activity less than 60% of wildtype
  - Note: this threshold is not 100% specific for transactivation (TA) activity and is complicated by the fact that TA activity will vary depend on many factors, for instance cell line that is used (HeLa, INS, MIN6 etc).
- Western blotting and indirect immunofluorescence for protein expression (specifically levels and nuclear and cytoplasmic localization, respectively).
  - Determining appropriate thresholds for protein expression is more difficult due to variability in results due to the complexity of the technique. Sample preparations, gel loading, transfer efficiency, specificity of the antibody, choice of internal control and inaccurate detection and quantification are some of the factors that can contribute to varying and inconsistent results. If a reduction in protein expression is seen by immunoblotting, then further testing by quantitative PCR (qPCR) is recommended in order to measure the mRNA level and assess whether a reduction in amount of protein is due to a reduced mRNA level.
- To use PS3_Supporting, functional study must have been performed on a transfected variant. If a study was performed on a cell line generated from a patient sample (and therefore contains the variant plus wild-type allele) does not count as PS3_Supporting.

Modification Type: Gene-specific,Strength

Instructions: See list of approved functional studies and guidelines for interpretation of data.

PS4

Original ACMG Summary

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.
Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0.

See manuscript for detailed guidance.
Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Strong

7 (seven) or more unrelated occurrences = Strong. Variant should meet PM2_Supporting in order to use PS4 at any level (careful review of gnomAD QC data may be necessary to assess whether variant is real or an artifact, especially if variant is in a polyC region). Phenotype of affected individuals must include diabetes, without clear evidence of an autoimmune etiology.

- One or more positive diabetes autoantibodies (IA-2A, ZnT8A+, GAD)[7],[8],[9],[10]
- Very low or negative C-peptide, defined as either fasting or non-fasting random C-peptide (<200pmol/L or 0.6ng/mL)[11],[12] or urinary C-peptide/creatinine ratio <0.2 nmol/mmol[8],[9]

Modification Type: Gene-specific,Strength

Moderate

4-6 unrelated occurrences = Moderate. Variant should meet PM2_Supporting in order to use PS4 at any level. Phenotype of affected individuals must include diabetes, without clear evidence of an autoimmune etiology.

- One or more positive diabetes autoantibodies (IA-2A, ZnT8A+, GAD)[7],[8],[9],[10]
- Very low or negative C-peptide, defined as either fasting or non-fasting random C-peptide (<200pmol/L or 0.6ng/mL)[11],[12] or urinary C-peptide/creatinine ratio <0.2 nmol/mmol[8],[9]

Modification Type: Gene-specific,Strength

Instructions: The phenotype of the patient must include diabetes, with evidence of an autoimmune etiology and/or absolute or near-absolute insulin deficiency (see above) considered as exclusionary. Variant should meet PM2_Supporting in order to use PS4 at any level (careful review of gnomAD QC data may be necessary to assess whether variant is real or an artifact, especially if variant is in a polyC region).

PM1

Original ACMG Summary

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Moderate

Applicable to amino acids that directly bind DNA and are necessary for Zinc-finger or homodimer formation[2]

- Directly bind DNA: Asp43, His49, Tyr 50, Gly51, Asp56, Gly57, Lys59, Arg63, Arg64, Arg67, His70, Tyr72, Arg87, Asn88, Arg91, Arg94, Gln109, Arg112
- Homodimer: Arg75, Gln89, Glu111, Asp113
- Zinc finger: Cys38, Cys41, Cys55, Cys58, Cys74, Cys80, Cys90, Cys93

Modification Type: Gene-specific,Strength

Supporting

This criterion can be used for missense variants in well-conserved regions within the DNA and ligand-binding domains. It can also be used for variants within certain transcription factor binding sites in the promoter (see below for details).

- Promoter region:
  - c.-132 to c.-151 (HNF6/OC2 and IPF1 binding sites)
  - c.-169 to c.-181 (HNF1A/HNF1B binding sites)
- DNA binding:
  - codons 37-113 (NM_175914.4:c.175C-339C p.Leu37-Asp113) (While the paper describing the crystal structure of *HNF4A*[2] shows the sequence as amino acids 33-113, amino acids 33-36 do not bind DNA and the conserved sequence starts as Leu37.)
- Ligand binding:
  - codons 180-220 and 300-350
    - (NM_175914.4:c.538G-658G p.Ala180-Val220)
    - (NM_175914.4:c.898T-1048G p.Tyr300-Glu350)

Modification Type: Gene-specific,Strength

PM2

Original ACMG Summary

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.
Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting

gnomAD 2.1.1 Popmax FAF ≤ 1:333,000 (≤ 0.000003 or 0.0003%) in gnomAD European Non-Finnish population AND ≤ 2 copies observed in ENF AND ≤ 1 copy in any other founder or non-founder population.

Modification General recommendation,Gene-specific

Type:

Instructions: Recommend using as supporting level of evidence (PM2_Supporting) per ClinGen guidance. Per guidance from ClinGen/SVI, PM2_Supporting + PVS1 is sufficient evidence of a variant being likely pathogenic. We recommend investigating the genotype metrics in gnomAD for variants that have been flagged for having failed one or more quality parameters, as it is possible that some of these filtered variants are actually real. The number of filtered alleles can be counted to determine whether PM2_Supporting would be met even if they were genuine calls. If the filtered calls are sufficient in number to not meet PM2_Supporting, then we would not use it. Because it is also possible that these calls are false positives, we would not use filtered variants to support BA1 or BS1.

PM3

Original ACMG Summary

For recessive disorders, detected in trans with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase.

Not Applicable

PM4

Original ACMG Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Moderate

For single amino acid deletions, use as supporting level of evidence.

Modification Type: Strength

Supporting

For single amino acid deletions/insertions, use as supporting level of evidence

Modification Type: Strength

PM5

Original ACMG Summary

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.
Example: Arg156His is pathogenic; now you observe Arg156Cys.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

Applicable once two amino acid changes have been classified as pathogenic at the same amino acid residue.

Modification Type: Strength

Moderate

The novel amino acid change must have a Grantham distance greater than or equal to the previously classified pathogenic variant.

Modification Type: Strength

Supporting

Apply if the previously classified amino acid change is likely pathogenic (rather than pathogenic) or if the previously classified variant is pathogenic but has a greater Grantham distance than the novel variant.

Modification Type: Strength

PM6

Original ACMG Summary

Assumed de novo, but without confirmation of paternity and maternity.

Not Applicable

Comments: Subsumed by PS2.

PP1

Original ACMG Summary

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.
Note: May be used as stronger evidence with increasing segregation data.

Strong

Use thresholds suggested by Jarvik and Browning[5]

- Single Family : ≤ 1/32 (5 meioses)
- > 1 Family : ≤ 1/16 (4 meioses)

Modification Type: General recommendation,Gene-specific

Moderate

Use thresholds suggested by Jarvik and Browning[5]

- Single Family : ≤ 1/16 (4 meioses)
- > 1 Family : ≤ 1/8 (3 meioses)

Modification Type: General recommendation,Gene-specific

Supporting

Use thresholds suggested by Jarvik and Browning[5]

- Single Family : ≤ 1/8 (3 meioses)
- > 1 Family : ≤ ¼ (2 meioses)

Modification Type: General recommendation,Gene-specific

Instructions: Variable penetrance and phenocopies complicate co-segregation studies. The presence of type 1 and type 2 diabetes phenocopies and significance of variants in unaffected individuals as defined above will need to be considered. If a family member(s) shows evidence of an autoimmune etiology for their diabetes and/or absolute or near-absolute insulin deficiency (see below), do not include them in PP1 calculation.One or more positive diabetes autoantibodies (IA-2A, ZnT8A+, GAD) (Ref 7,8,9,10). Very low or negative C-peptide, defined as either fasting or non-fasting random C-peptide (<200pmol/L or 0.6ng/mL) (Ref 11,12) or urinary C-peptide/creatinine ratio <0.2 nmol/mmol (Ref 8,9). Unaffected family members without the variant under assessment can also be used in segregation analysis(Ref 5). An individual is considered “unaffected” if over age 70 and non-diabetic (based on Exeter work (Ref 13) which shows penetrance of HNF4A-MODY at 98% by age 70).

PP2

Original ACMG Summary

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

Not Applicable

Comments: While missense variants in HNF4A are a common mechanism of monogenic diabetes, and the constraint score for HNF4A (gene) is 1.81, the MDEP does not support using this criterion at this time.

PP3

Original ACMG Summary

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Supporting

Use REVEL score of ≥0.70 as supportive evidence of pathogenicity. We also support using SpliceAI to assess the predicted impact of non-canonical splicing variants and synonymous variants: apply PP3 when the predicted change is at least 0.2 [4,3].

Modification Type: General recommendation

PP4

Original ACMG Summary

Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

Moderate

Phenotype: MODY Probability Calculator result ≥50% chance of testing positive[6] AND negative *HNF1A* testing AND presence of at least one additional feature characteristic of _*HNF4A*_-MODY:

- Antibody negative and/or persistent C-peptide after five years following T1DM diagnosis
- Personal or family history of persistent neonatal hypoglycemia
- Personal or family history of large for gestational age (LGA) infants or macrosomia in the absence of sufficient maternal hyperglycemia
- Response to low-dose SU (extreme response- hypoglycemia)
- Biochemical/Molecular phenotypic evidence from patient cell lines
- Fanconi phenotype in conjunction with c.187C>T p.R63W

Modification Type: Gene-specific

Supporting

MODY Probability Calculator (MPC)[6] result ≥50% chance of testing positive AND negative *HNF1A* testing

Modification Type: Gene-specific

Instructions: MODY probability calculator result of ≥50% chance of testing positive (https://www.diabetesgenes.org/mody-probability-calculator/) AND negative HNF1A genetic analysis, given the similarities in phenotypes between HNF1A-MODY and HNF4A-MODY. Clinical judgement may need to be used when applying this criterion, as the MODY Probability Calculator is not as reliable for non-European ancestry individuals or people diagnosed >35. For example, use of PP4 is acceptable in the absence of HNF1A analysis when the MPC is >50% and the phenotype is specific to HNF4A, for example someone in the family with neonatal hypoglycemia that is responsive to diazoxide or hyperinsulinemic hypoglycemia. If individual was tested due to neonatal hypoglycemia, PP4 can be used if ABCC8 and KCNJ11 testing are negative (no MODY Probability Calculator result required). Certain assumptions can be made in order to use the MODY probability calculator. Specific clinical info about parents not given but lab/literature states “Family history of diabetes”, click “Parent with diabetes” in calculator. If no information about family history of diabetes is provided, run the calculator in both conditions (yes/no) and document whether this makes a difference in overall probability score. Weight/Height/BMI not given but lab/literature states patient is “lean”, enter BMI of 30. HbA1c is not provided, enter 6% and 10% and document whether this makes a difference in overall probability score. Treatment information is not provided, cannot use calculator.

PP5

Original ACMG Summary

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BA1

Original ACMG Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome

Aggregation Consortium.

Stand Alone

gnomAD 2.1.1 Popmax Filtering AF ≥ 1:10,000 (≥ 0.01% or 0.0001).

Modification Type: Gene-specific

Instructions: If there is a Popmax Filtering AF for both exomes and genomes, use the one with the larger denominator.

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

gnomAD 2.1.1 Popmax Filtering AF ≥ 1:30,000 (≥0.0033% or 0.000033).

Modification Type: Gene-specific

Instructions: If there is a Popmax Filtering AF for both exomes and genomes, use the one with the larger denominator.

BS2

Original ACMG Summary

Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.

Strong

Apply to normoglycemic individuals age 70 or older (i.e., genotype positive, phenotype negative)

Modification Type: Gene-specific

BS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Strong

Applicable to non-canonical splice site variants that have RNA and in silico evidence of normal splicing.

Modification Type: Gene-specific

Supporting

List of approved functional studies and guidelines for interpretation of data.

- EMSA for DNA binding
  - “No functional impact” is defined as ≥75% activity of wildtype
  - Note: the effect of the variant on DNA binding will be highly dependent on whether the variant is located within the DNA binding domain.
- Luciferase assays for transactivation
  - “No functional impact” is defined as ≥75% activity of wildtype
  - Note: this threshold is not 100% specific for transactivation (TA) activity and is complicated by the fact that TA activity will vary depend on many factors, for instance cell line that is used (HeLa, INS, MIN6 etc). Assays should include controls for WT, T2DM and known MODY variants.
- Western blotting and indirect immunofluorescence for protein expression (specifically levels and nuclear and cytoplasmic localization, respectively).
  - Determining appropriate thresholds for protein expression is more difficult due to variability in results due to the complexity of the technique. Sample preparations, gel loading, transfer efficiency, specificity of the antibody, choice of internal control and inaccurate detection and quantification are some of the factors that can contribute to varying and inconsistent results. If a difference in protein expression compared to WT is seen by immunoblotting, then further testing by quantitative PCR (qPCR) is recommended in order to measure the mRNA level and assess whether the difference in amount of protein is due to a reduced mRNA level.

Modification Type: Gene-specific

Instructions: To use BS3, functional study must have been performed on a transfected variant. If a study was performed on a cell line generated from a patient sample (and therefore contains the variant plus wild-type allele) it cannot count as BS3.

BS4

Original ACMG Summary

Lack of segregation in affected members of a family.
Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Strong

Applicable to family members without variant who have MPC[6] score ≥50% (i.e., genotype negative, phenotype positive).

Modification Type: General recommendation,Gene-specific

BP1

Original ACMG Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Not Applicable

BP2

Original ACMG Summary

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

Supporting

Also applicable when in cis or trans with a likely pathogenic variant.

Modification Type: General recommendation

BP3

Original ACMG Summary

In frame-deletions/insertions in a repetitive region without a known function.

Not Applicable

BP4

Original ACMG

Summary

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Supporting

Use a REVEL score of ≤0.15 as supportive evidence of no predicted impact on the gene or gene product. We also support using SpliceAI to assess the predicted impact of non-canonical splicing variants and synonymous variants: apply BP4 when the predicted change is below 0.2[3],[4].

Modification Type: General recommendation

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Supporting

A variant in another monogenic diabetes gene is Pathogenic/Likely Pathogenic.

Modification Type: General recommendation

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BP7

Original ACMG Summary

A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Supporting

Apply BP7 when the predicted change from SpliceAI is below 0.2 AND phyloP100 way < 2.0.

Modification Type: Gene-specific

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)*

1 Very Strong *(PVS1, PS2_Very Strong)* **AND ≥ 2 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)* **AND 1 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Very Strong *(PVS1, PS2_Very Strong)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)*

≥ 2 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)*

Likely Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)*

≥ 3 Moderate *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)*

2 Moderate *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Moderate *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting,*

PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM5_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS2_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong)* **AND ≥ 1 Supporting** *(PVS1_Supporting, PS1_Supporting, PS2_Supporting, PS3_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PM5_Supporting, PP1, PP3, PP4)*

Benign

≥ 2 Strong *(BS1, BS2, BS3, BS4)*

1 Stand Alone *(BA1)*

Likely Benign

1 Strong *(BS1, BS2, BS3, BS4)* **AND 1 Supporting** *(BS3_Supporting, BP2, BP4, BP5, BP7)*

≥ 2 Supporting *(BS3_Supporting, BP2, BP4, BP5, BP7)*

Files & Images

HNF4A PVS1 Decision Tree: Decision tree for determining the strength of PVS1 to apply to variants in HNF4A for monogenic diabetes

PS2 De Novo Points Table: Table for determining strength of PS2 for apparent de novo variants

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